

The University of Chicago

IN VITRO AND IN VIVO EXPERI-
MENTS ON THE DIGESTIBILITY
OF RAW AND HEAT-TREATED
EGG WHITE

A PART OF A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIOLOGICAL
SCIENCES IN CANDIDACY FOR THE DEGREE
OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF HOME ECONOMICS AND HOUSEHOLD
ADMINISTRATION

1936

By
ESSIE WHITE COHN

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PART I
THE ENZYMATIC HYDROLYSIS OF RAW AND
HEAT-TREATED EGG WHITE

The effect of a preliminary heat treatment of proteins upon their digestion in vitro appears to be dependent to some degree on the nature of the protein. The studies of Waterman and Johns (24) and of Jones and Waterman (12) have demonstrated that a partial cooking of phaseolin, casein, and cottonseed globulin increases the extent of hydrolysis of these proteins by the successive action of pepsin and trypsin. The digestion of arachin, however, was uninfluenced by heat treatment prior to enzymatic action.

The question of the relative physiological value of raw and of heat-treated egg white has served to emphasize again the importance of heat as a factor in determining the extent of utilization of proteins. Investigations dealing with this subject have in the majority of instances involved growth or nitrogen balance studies; conflicting results have been reported (Bateman, 4; Boas, 7; Friedberger and Seidenberg, 10; Mendel and Lewis, 15; Rose and MacLeod, 21). Of particular interest are the recent studies of Parsons and coworkers (17, 18, 19) and of Salmon and Goodman (22) on the nutritional disorder produced by feeding large quantities of raw egg white to rats.

In view of the conflicting results and diverse explanations concerning egg white in nutrition, an investigation of the in vitro hydrolysis of raw and of heat-treated egg white has been conducted. Data have been obtained concerning: (1) the comparative rates of digestion of raw and of heat-treated egg white in vitro, and (2) the extent of hydrolysis of both types of substrate by pepsin and trypsin acting both individually and successively.

Experimental

A stock egg white powder was prepared by drying the whites of fresh eggs in vacuo over sulfuric acid at 25°; the dried product was ground to a fine powder and thoroughly mixed. Standard egg white solutions were prepared by suspending 5 gm. of the powder in 500 cc. of water, shaking until a uniform suspension was obtained, and then slowly adding with shaking 450 cc. of boiling distilled water. The mixture was thoroughly agitated until solution was practically complete. (A small, barely perceptible amount of material failed to dissolve.) The solution was cooled to room temperature, and diluted to 1000 cc. with distilled water. One hundred cubic centimeter aliquots were withdrawn; for purposes of reference, these will be called Samples A, B, C, D, etc.

Aliquot Sample A was adjusted to pH 8.0 with 1 per cent sodium hydroxide, warmed on a water bath to 40°, and treated with 10 cc. of a 1 per cent trypsin solution. (A 1:110 trypsin from the Difco Laboratories was used.) The flask was shaken thoroughly and a 10 cc. portion withdrawn for analysis. To the remainder of the solution were added 5 cc. of a 0.4 per cent thymol solution in ethyl alcohol and the flask was stoppered and placed in an incubator maintained at 38°. Samples were withdrawn at varying intervals of time for analysis. Enzymatic action in each withdrawn aliquot was terminated by heating the solution for 5 minutes at 80°. The sample was then allowed to cool to room temperature and titrated by means of a Sørensen formol titration (23).

A series of check determinations has been conducted in which the increase in carboxyl groups was determined by titration in 90 per cent alcohol according to the method of Willstätter and Waldschmidt-Leitz (25), with thymolphthalein as the indicator. The trend of the values given by the latter procedure was similar to that yielded by the Sørensen method. Since the results in these experiments are of interest because of their relative, rather than their absolute magnitude, the titration values obtained with the Sørensen technique are given here. The significance of the measurements by each of these two volumetric methods, and the adequate accuracy of the Sørensen procedure for the present type of investigation, have recently received excellent discussion by Richardson (20).

A second 100 cc. aliquot (Sample B) of the egg white solution was immersed in a boiling water bath for exactly 1.5 minutes. (The egg white solution became slightly opalescent when subjected to the heat treatment, but there was no flocculation of protein.) In a similar manner, subsequent aliquots (Samples C, D, E, etc.) were heated for 5, 10, 30, 45, 60, and 120 minutes respectively. Each of these solutions was then made up to the original 100 cc., replacing the water lost by evaporation during the boiling process. These aliquots were then treated with trypsin, incubated, and analyzed at intervals, as described above. For each 100 cc. aliquot, simultaneous control experiments were performed by incubation of: (1) 0.1 gm. of trypsin dissolved in 100 cc. of water and (2) 100 cc. of stock egg white solution; both controls were adjusted to pH 8.0, and analyzed at the same intervals as were the withdrawn samples of the experimental digest. The sum of the two titration values from these control solutions was considered as a blank and subtracted from the figure obtained by analysis of the digest aliquot. Table I presents the experimental values of these trypsin studies.

TABLE I
HYDROLYSIS OF EGG WHITE BY TRYPSIN

Each value under the head "Incubation time, in min." represents the corrected cc. of N/10 NaOH required per gram of nitrogen.*

Preliminary Heating Period	Incubation Time, in Min.							
	20	40	60	90	120	180	240	300
Min.								
0	20.1	20.1	20.1	24.2	26.1	26.1	30.1	32.1
1.5	32.1	44.1	46.1	54.1	62.2	70.3	76.2	86.3
5.0	58.4	80.5	86.4	102.8	112.9	124.8	140.9	145.0
30.0	117.5	131.6	149.2	151.3	163.8	175.6	181.0	191.0

*The above values were determined from the titration figures obtained on 10 cc. aliquots of egg white solution containing 0.5 gm. dry egg white per 100 cc. of solution. The titration figures were in cc. of N/10 NaOH required to neutralize the acids liberated by the action of trypsin upon the egg white substrate. The concentration of trypsin was 0.1 per cent. The amino groups were blocked and the carboxyls determined by means of Sørensen's (23) formol titration.

Experiments were also conducted in which the action of trypsin was preceded by an incubation period with pepsin. In these

experiments, 100 cc. aliquots (Samples A, B, C, etc.) of a stock solution of 0.5 per cent egg white powder in 0.3 per cent hydrochloric acid was used. Sample A was treated with 10 cc. of a 1 per cent solution of pepsin (1:3000 pepsin, Parke, Davis and Company) preserved with thymol, and incubated at 38° for 90 minutes. At the end of this period, a 10 cc. aliquot was withdrawn for analysis; the remaining contents of the flask were adjusted to pH 8.0 with N sodium hydroxide and treated with trypsin, incubated, and analyzed at intervals as already described. Simultaneous blank determinations were conducted on egg white in 0.3 per cent hydrochloric acid and on the enzyme solutions. The experimental values obtained were corrected by these blank titration values.

Aliquot Samples B, C, D, etc., were subjected to preliminary heating periods of varying length, before being treated with pepsin. The subsequent procedure was identical with that outlined in the preceding paragraph. The results of the studies in which trypsin was preceded by pepsin are presented in Table II.

TABLE II

HYDROLYSIS OF EGG WHITE BY TRYPSIN FOLLOWING A 90 MINUTE
INCUBATION PERIOD WITH PEPSIN

Each value represents the corrected cc. of N/10 sodium hydroxide required per gram of nitrogen.*

Preliminary Heating Period	Incubation Time, in Min.							
	20	40	60	90	120	180	240	300
Min.								
0	92.9	115.2	127.2	145.5	148.7	170.5	176.8	176.8
1.5	108.8	127.2	131.1	139.5	167.0	176.8	176.8	186.5
5.0	146.2	150.0	163.5	164.5	177.2	179.2	191.0	195.0
30.0	152.1	162.0	177.2	169.0	199.0	204.5	208.5	220.5
45.0	177.2	196.2	210.5	222.2	222.2	243.5	249.0	253.5

*The values have been obtained by subtraction of the titration figure found at the end of the 90 minute incubation period for pepsin from the titration value resulting after tryptic incubation. These pepsin values, expressed as cc. of N/10 sodium hydroxide per gram of nitrogen, were 0.0 cc. for raw egg white, and 28.2, 42.2, 70.4, and 150.0 cc. for egg white subjected to preliminary heating periods of 1.5, 5, 30, and 45 minutes respectively

Under the experimental conditions employed, the cleavage of raw egg white by pepsin is negligible, while trypsin exhibits

a limited ability to hydrolyze this substrate. However, despite the apparent inactivity of pepsin with the raw egg white, it appears that this enzyme did cause some change in this material, since tryptic action was much more extensive when preceded by pepsin than when trypsin alone was used (compare Table I and Table II, raw egg white).

A preliminary heating of the egg white solution prior to incubation with the enzymes resulted in a marked increase in the extent of hydrolysis of the substrate. When trypsin alone was used, the degree of hydrolysis of egg white increased as the preliminary heating period was extended to 30 minutes. Longer heating periods did not further the hydrolytic process; the titration data for egg white solutions heated for 30, 45, 60, or 120 minutes previous to incubation with trypsin are identical within the limits of experimental error. When the action of trypsin was preceded by pepsin, there was a continued increase in the magnitude of hydrolysis of egg white as the preliminary cooking period lengthened to 45 minutes. Egg white solutions heated for 45, 60, or 120 minutes previous to peptic plus tryptic action yielded similar data.

Discussion

The results which have been obtained are in harmony with those of in vivo experiments which have indicated a better utilization of heat-treated than of raw egg white. Of the hypotheses which have been offered to explain this difference in physiological availability, the suggestion that the indigestibility of raw egg white is due to a heat-labile, antitryptic factor appears to be in concordance with the present findings. The recent work of Balls and Swenson (3) on the antitrypsin of egg white is of considerable interest in this connection. These investigators reported the preparation from egg white of a concentrate which inhibits the proteolytic activity of trypsin; the product was described as moderately heat-stable and destroyed by continued boiling. It appears quite possible that under the experimental conditions herein employed the shorter boiling intervals brought about a partial inhibition of the antitrypsin of egg white, while the longer periods of cooking effected a complete destruction of this antienzyme. It is likely, however, that the reported poor utilization and evident toxicity of raw egg white is not due solely to an antitrypsin. The physical characteristics of the material per se may be a contribut-

ing factor. It seems significant that the lack of nutritional value of the uncooked material may be related to the limited time which it remains in the stomach (Beaumont, 5; Cannon, 8; London and Sulima, 13). This shorter period would tend to restrict the action of pepsin; the important role of this gastric enzyme in the proteolysis of egg white is indicated by the results given here. In fact, a preliminary treatment with pepsin compares favorably with a 30-minute period of cooking of the egg white in its ability to augment the action of trypsin (compare Table I, 30-minute preliminary heating period, with Table II, no preliminary heating period).

PART II

THE DIGESTION OF THIN AND THICK EGG WHITE

That egg albumin possesses an antitryptic influence has been suggested by a number of workers (Abderhalden and Pettibone, 1; Bateman, 4; Bizarro, 6; Cohnheim, 9; Hedin, 11; Long and Johnson, 14). This antitryptic factor appeared to be destroyed by heating to 70° or above. More recently Balls and Swenson (3) have given evidence that there exists an antitryptic factor in egg white and have indicated means by which this substance may be partially concentrated. They have stated that this antitryptic material is present in active form almost entirely within the thin, watery fraction of egg white (2). These latter experiments were conducted by a study of the hydrolysis of casein after the addition of an antitryptic concentrate which was separated from the watery egg white. From the results given in the preceding section, and a consideration of the literature, it appeared likely that the digestibility of raw egg white by trypsin, after the preliminary action of pepsin, was due to destruction, without hydrolysis, of the antitryptic factor. Since Balls and Swenson had found the thin egg white to be the source of the antitryptic material, the following experiment was indicated: (1) to digest by means of trypsin alone the thin fraction of raw egg white, (2) to digest by trypsin alone the thin fraction of egg white which had been subjected to a preliminary five-minute heat treatment, (3) to subject to tryptic action the thin fraction of egg white which had been previously exposed to pepsin, (4) to perform the same series of experiments as just indicated, only using the thick fraction of egg white.

Experimental

The experimental methods used were similar to those indicated in Part I. The results themselves are presented in tabular form in Tables III and IV.

From the data given in Tables III and IV it is evident that there is but slight difference in the hydrolysis of unheated thin and thick egg white, incubated with trypsin alone. It is

TABLE III
DIGESTION OF THIN EGG WHITE EXPRESSED IN
cc. N/10 NaOH PER GRAM OF NITROGEN

Time in Minutes	(1) ^a	(2) ^b	(3) ^c
	Hydrolysis of Unheated Egg White	Hydrolysis of 5 Minute Heated Egg White	Hydrolysis of Unheated Egg White
0	0	0	0
20	8.1	32.2	31.2
40	8.1	46.8	60.4
60	10.8	58.6	69.8
90	18.1	64.7	87.35
120	24.4	66.6	96.2
180	32.2	72.6	98.2
240	38.6	78.6	114.8

^aUnheated egg white digested by trypsin only.

^bEgg white subjected to five-minute preliminary heat treatment followed by trypsin alone.

^cUnheated egg white digested for ninety minutes by pepsin followed by the trypsin digest as indicated.

TABLE IV
DIGESTION OF THICK EGG WHITE EXPRESSED
IN cc. N/10 NaOH PER GRAM OF NITROGEN

Time in Minutes	(1) ^a	(2) ^b	(3) ^c
	Hydrolysis of Unheated Egg White	Hydrolysis of 5 Minute Heated Egg White	Hydrolysis of Unheated Egg White
0	0	0	0
20	10.2	45.2	30.6
40	12.3	49.8	51.2
60	20.5	67.8	53.4
90	28.7	76.1	70.1
120	37.3	82.2	76.0
180	41.1	92.5	80.2
240	45.3	102.5	82.4

^aUnheated egg white digested by trypsin only.

^bEgg white subjected to five-minute preliminary heat treatment followed by trypsin alone.

^cUnheated egg white digested for ninety minutes by pepsin followed by the trypsin digest as indicated.

evident that the hydrolysis of thick egg white which has undergone a five-minute heat treatment prior to incubation with trypsin shows a greater hydrolysis than does the thin egg white under the same conditions of treatment. The results also indicate that when thin and thick egg white have been incubated first with pepsin and then with trypsin, the action of pepsin upon the thick white is not as great as is its action upon the thin egg white substrate.

Discussion

The following inferences appear to be logical from the results given in Tables III and IV: (1) heat treatment and exposure to pepsin both will cause an increased digestibility of either thin or thick egg white, (2) if the antitryptic factor resides absolutely in the thin portion of egg white, the increased action is not due to the destruction of the antitryptic factor, (3) if the increased action due to pepsin or heat is due to the destruction of the antitryptic factor, then the small quantity of the antitryptic factor in the thick portion of egg white has almost the same influence in retardation of tryptic hydrolysis as does the greater amount of inhibitor in the thin portion.

PART III
ANIMAL EXPERIMENTS WITH EGG WHITE

It was found by Parsons (17) that when rats are fed a diet containing 66 per cent of raw egg white, the diet being normal in other respects, these animals will develop a dermatitis condition. It was thought, in view of the experiments performed, that the material which reduced the digestibility of egg white in vitro might be the toxic factor in vivo. If this were the case, this so-called antitryptic material should be of protein nature because the preceding work had indicated that it was denatured by the action of pepsin. Therefore, a program involving the injection of egg white in increasing dosages into the rat was conducted prior to the feeding of an egg white diet. The purpose of this experiment was to determine whether the preliminary injection of egg white would tend to affect the rat, because either sensitization or immunization would indicate that the toxic material was protein in nature. Since Parsons (18) had found that heat treatment rendered the egg white innocuous, it would be the protein material which possessed the ability to limit the action by trypsin which would be responsible for the dermatitis condition; in other words, the so-called "antitrypsin."

Experimental

The rats were injected three times a week for a period of eight weeks. During the period of the injections they were fed a basal ad libitum diet (diet a) consisting of: casein 18, salt mixture 4, sucrose 15, starch 38, and lard 25. This diet was supplemented by a daily ration to each animal of 400 mg. of dried yeast and 4 drops of cod liver oil. Every other day the animal was given a minimum of 3 gm. of lettuce.

The rats were then placed upon the 66 per cent egg white diet (diet d) used by Parsons and Kelly (18). This diet consisted of: dry egg white 66, salt mixture 4, yeast 10, wheat embryo 10, sucrose 10; also, a daily supplement to each animal of 4 drops of cod liver oil. During the last week of the standard diet and the first week of the egg white diet, dried beef liver was added to

the diets to prevent the nutritional disorder observed by Parsons in young rats when the liver was not added. The transition diet when 2 gm. of liver was added daily to diet a is called diet b; whereas, the transition diet when one gram of liver was added to diet d is called diet c. After the rats were placed upon the egg white diet, three injections of egg white were given, one each week. The concentration of the egg white injections is shown in Table V.

The weights of the animals were recorded every other day throughout the experiments. There were four groups of animals of five animals each. The first group were male controls; the second, male rats which were injected as indicated in Table V. These animals were chosen so that the average weight of each group was approximately the same. The third group were female controls; the fourth, females, injected as indicated in Table V. Here again, the average weight of the animals within the last two groups was approximately alike.

TABLE V
EGG WHITE INJECTIONS

Day	Concentration of Egg White	Day	Concentration of Egg White
1st	1:1,000,000	31st	1:7,000
3rd	1:1,000,000	33rd	1:4,000
5th	1:500,000	35th	1:2,000
7th	1:500,000	38th	1:1,000
10th	1:250,000	40th	1:700
12th	1:100,000	42nd	1:400
14th	1:75,000	45th	1:200
17th	1:50,000	47th	1:100
19th	1:35,000	49th	1:80
21st	1:35,000	51st	1:50
24th	1:20,000	53rd	1:20
26th	1:15,000	55th	1:10
28th	1:10,000	58th	1:10

During the course of the experiment, the animals were examined for the following types of symptoms which would indicate their condition: eye symptoms, tail symptoms, and fur condition. To obtain a semi-quantitative measure of the degree of the severity of the dermatitis, a symptom index was devised. The index consisted of a number which was the sum of a group of numbers which

represented the degree of severity of the individual symptom. The numbers were assigned as follows: eye condition: slightly watery, 1; watery, 2; very watery, 3; slightly scaly, 4; scaly, 5; very scaly, 6. Tail: soiled, 1; slightly scaly, 2; scaly, 3; very scaly, 4. Fur: slightly rough, 1; rough, 2; very rough, 3. Since the sum of the numbers which would represent the most severe symptom would be 13, a symptom number of 13 would represent a rat which was most severely affected by the egg white diet; whereas, a symptom number of zero would represent a healthy animal.

The average of weights and the symptom numbers of the rats in the experiment and a listing of the diets are given in Table VI.

TABLE VI
AVERAGE OF WEIGHTS AND SYMPTOMS OF
INJECTED AND NON-INJECTED RATS

Week	Diet	Non-Injected Male Rats		Injected Male Rats		Non-Injected Female Rats		Injected Female Rats	
		Wt. in Gm.	Symp- tom No.	Wt. in Gm.	Symp- tom No.	Wt. in Gm.	Symp- tom No.	Wt. in Gm.	Symp- tom No.
0	a	64	0	62	0	55	0	54	0
1	a	82	0	78	0	70	0	67	0
2	a	96	0	92	0	83	0	81	0
3	a	122	0	113	0	101	0	98	0
4	a	146	0	134	0	116	0	116	0
5	a	166	0	161	0	129	0	123	0
6	a	188	0	178	0	138	0	137	0
7	b	197	0	192	0	147	0	143	0
8	c	202	0	198	0	152	0	146	0
9	d	203	0	198	0	155	0	146	0
10	d	205	0	197	0	159	0	148	0
11	d	213	4.3	205	6.8	168	6.3	155	6.3
12	d	218	6.5	207	7.0	170	6.8	161	7.5
13	d	227	6.6	221	6.8	174	7.1	164	7.7
14	d	232	7.0	226	7.3	177	6.6	166	7.2
15	d	235	6.9	231	6.9	179	6.2	168	8.0
16	d	237	7.5	235	7.5	179	6.7	169	8.6
17	a	238	7.4	238	7.5	178	7.5	170	9.6
18	a	252	7.5	244	7.3	188	6.8	177	8.9
19	a	270	7.8	268	7.1	196	6.7	184	9.0
20	a	278	6.3	277	6.3	198	4.4	182	6.5
21	a	291	4.7	285	5.8	201	3.5	185	6.3
22	a	290	3.8	285	4.4	197	3.6	183	3.2
23	a	295	3.7	288	3.8	200	2.7	186	4.1

The results given in the table are indicated in graphical form in Figure 1. In this figure, Curve A presents the values of the weights in the case of the male animals. The solid line represents the weights of the non-injected animals, whereas the dotted line represents the weights of the injected animals. In Curve B of the figure, the weights of the non-injected females and the injected female animals are presented in solid and broken lines respectively. In Curve C are graphed to the Symptom Number scale the average symptom numbers of both male and female groups. The full line represents the Symptom Numbers for the non-injected group and the dotted line represents the Symptom Numbers of the injected groups.

From these results it is evident that the weights of the injected rats were less in the case of both male and female groups than the weights of the non-injected groups, under the same conditions of diet. The differences became more pronounced after the egg white feeding was commenced. The egg white feeding produced a diminution in the rate of growth in the case of both male and female groups, which is made very evident by the rapid increase of growth when the basal diet was resumed. Curve C indicates in a striking way that when the animals were again placed upon a standard diet, after the feeding of egg white, the symptoms were diminished. The diminution in symptom was proportional to the increase in weight, except for the fact that the weight began to improve almost immediately whereas a two-week time interval elapsed before the symptoms began to diminish. It will be noted that whereas the non-injected groups were of the higher weight, the non-injected groups were of the lower Symptom Numbers.

Discussion

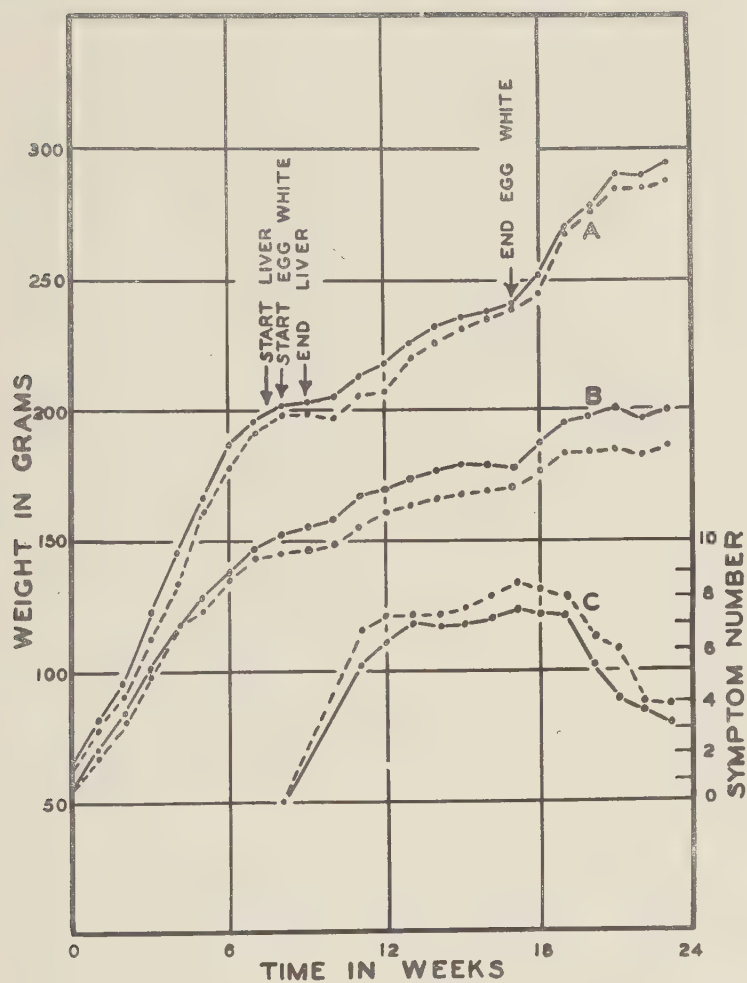
From the results obtained it is evident that a sensitization has been produced in the rats by the injection of egg white previous to the feeding of the egg white diet. Upon the basis of the argument given in the introduction of this section, it is inferred that the antitrypsin material of Balls and Swenson is protein in nature and is probably responsible for the dermatitis condition observed when egg white is fed to rats. That sensitization of any kind is produced is of interest as it is well known that it is very difficult to sensitize rats.

Fig. 1.--Symptom Numbers of Rats, Presented with Average Growth Curves of Injected and Non-injected Male Rats and with Injected and Non-injected Female Rats.

Curves A, solid line represents the average growth curve of non-injected male rats; broken line represents the average growth curve of injected male rats.

Curves B, solid line represents the average growth curve of non-injected female rats; broken line represents the average growth curve of injected female rats.

Curves C, solid line represents average symptom number of male and female non-injected rats; broken line represents average symptom number of male and female injected rats.



Summary

1. Pepsin produced no significant splitting of raw egg white under the experimental conditions employed. The hydrolysis effected by trypsin was slight; a preliminary incubation of the raw egg white with pepsin greatly facilitated the attack of the material by trypsin.
2. Preliminary periods of heating, up to 30 minutes in length, increased the degree of hydrolysis of egg white by trypsin. The extent of digestion of egg white by the successive action of pepsin and trypsin also varied directly with the length of the preliminary heating period to which the substrate was subjected.
3. These experimental results are interpreted as supporting the existence in raw egg white of an antitryptic agent which is slowly inactivated by heat.
4. There is a slight difference in the hydrolysis of unheated thin and thick egg white which has been incubated with trypsin alone.
5. Thick egg white which has undergone a five-minute heat treatment prior to incubation with trypsin shows greater hydrolysis than does thin egg white which has been submitted to the same heat treatment.
6. Pepsin, on unheated egg white, shows a more marked action on thick egg white than it does on thin egg white.
7. Growth curves of rats on an egg white diet have been obtained both for rats previously injected with egg white and those not treated in this manner.
8. It was found that the average weight of the non-injected animals in both the male and female groups was greater than the weight of the injected animals.
9. The growth of animals on the egg white diet fell below their rate of growth on the standard diet which was evidenced by the fact that when replaced upon a standard diet, the rate of growth was markedly accelerated.
10. A symptom index has been established which allows of a semi-quantitative expression of the degree of ill health. The symptom index of previously injected animals is higher than for the non-injected group.

11. There is a lag of improvement in condition of animals behind the rate of growth after the animals are removed from the egg white diet.

12. From the animal experiments, the conclusion is drawn that the material responsible for a dermatitis condition is a protein which appears to give the same response as might be expected from the antitryptic factor of egg white.

BIBLIOGRAPHY

1. Abderhalden, E., and Pettibone, C.J.V., Z. Physiol. Chem., 81, 458 (1912).
2. Balls, A.K., and Swenson, T.L., J. Ind. Eng. Chem., 26, 570 (1934).
3. Balls, A.K., and Swenson, T.L., J. Biol. Chem., 106, 409 (1934).
4. Bateman, W.G., J. Biol. Chem., 26, 263 (1916).
5. Beaumont, W., Experiments and observations on the gastric juice and the physiology of digestion, Plattsburgh (1833).
6. Bizarro, A.H., J. Physiol., 46, 267 (1913).
7. Boas, M.A., Biochem. J., 21, 712 (1927).
8. Cannon, W.G., Am. J. Physiol., 12, 387 (1904-05).
9. Cohnheim, O., "Enzymes," John Wiley and Sons, New York, 1912.
10. Friedberger, E., and Seidenberg, S., Deutsch. med. Woch., 53, 1507 (1927).
11. Hedin, S.G., Z. physiol. Chem., 52, 412 (1907).
12. Jones, D.B., and Waterman, H.C., J. Biol. Chem., 52, 357 (1922).
13. London, E.S., and Sulima, A.T., Z. physiol. Chem., 46, 209 (1905).
14. Long, J.H., and Johnson, W.A., J. Amer. Chem. Soc., 35, 1188 (1913).
15. Mendel, L.B., and Lewis, R.C., J. Biol. Chem., 16, 55 (1913-14).
16. Northrop, J.H., J. Gen. Physiol., 9, 767 (1925-26).
17. Parsons, H.T., J. Biol. Chem., 90, 351 (1931).
18. Parsons, H.T., and Kelly, E., J. Biol. Chem., 100, 645 (1933); Am. J. Physiol., 104, 150 (1933).
19. Parsons, H.T., and Lease, J.G., J. Nutrition, 8, 57 (1934).
20. Richardson, G.M., Proc. Roy. Soc. London, Series B, 115, 121, 142 (1934).
21. Rose, M.S., and MacLeod, G., J. Biol. Chem., 50, 83 (1922).

22. Salmon, W.D., and Goodman, J.G., J. Nutrition, 8, 1 (1934).
23. Sørensen, S.P.L., Biochem. Z., 7, 45 (1908).
24. Waterman, H.C., and Johns, C.O., J. Biol. Chem., 46, 9 (1921).
25. Willstätter, R., and Waldschmidt-Leitz, E., Ber. chem. Ges.,
54, 2988 (1921).

